Enterococci by Membrane Filtration using mEI Agar
EPA 1600 – December 2009

Facility Name:_____ LAB ID:_____

Assessor Name:_____ Analyst Name:_____ Inspection Date:_____

Records Examined: SOP Number/Revision/Date:_____ Analyst:_____

Sample ID:_____ Date of Sample Preparation:_____ Date of Analysis:_____

Relevant Aspect of Standards	Method Reference	Y	N	N/A	Comments
1) Is the maximum sample holding time 8 hours?	40CFR136.3(e) Table II				
2) Are pipets sterile and made of glass or plastic and of appropriate volume?	6.5				
3) Are membrane filter units sterilized and kept wrapped in foil or kraft paper?	6.7				
4) Are membrane filters, sterile, white grid marked, 47mm diameter, w/ 0.45 ± 0.02µm pore size used?	6.20				
5) Is the sample incubator maintained at 41 ± 0.5˚C?	6.22				
6) Is the waterbath for tempering agar maintained at 50˚C?	6.23				
7) Is phosphate buffered saline (PBS) made as follows? Sodium dihydrogen phosphate (NaH_2PO_4)…0.58 g Disodium hydrogen phosphate (Na_2HPO_4)…2.5 g Sodium chloride (NaCl)……..8.5 g Reagent-grade water…….1.0 L	7.4.1				
8) Is prepared PBS autoclaved at 121°C for 15 min? Is final pH 7.4 ± 0.2?	7.4.2				
9) Is the modified mEI Agar prepared by adding 72 g dehydrated powder to 1 L reagent-grade water, mixed and heated until dissolved, autoclaved at 121°C for 15 min, and cooled in a 50°C water bath? [NOTE: Check composition of agar against specifications in 7.5.1]	7.5.2				
10) After sterilization and tempering, is 0.24 g nalidixic acid (sodium salt) and 0.02 g triphenyltetrazolium chloride (TTC) added to mEI agar and mixed thoroughly?	7.5.3				

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11) Is approximately 4-6 mL of mEI agar poured into 9x50 mm or 15x60 mm petri plates to a depth of 4-5 mm, allowed to solidify and stored in the refrigerator?	7.5.4				
12) Is the final pH of the agar 7.1 ± 0.2?	7.5.4				
13) Is a filtration blank performed by filtering and plating on Tryptic Soy Agar a 50-mL volume of sterile PBS before beginning sample filtrations? Is the blank incubated for 24 ± 2 hours at 35°C ± 0.5°C?	9.10				
14) Is each petri dish and report form marked with the sample identification and volume?	11.2				
15) Is a sterile membrane filter placed on the filter base grid side up with sterile smooth-tipped forceps, and funnel attached to the base with membrane filter held between the funnel and the base?	6.13 11.3				
16) Is the sample shaken vigorously at least 25 times?	11.4				
17) Are sample volumes chosen to produce 20-60 enterococci colonies? *It is recommended that a minimum of 3 dilutions be analyzed to ensure that a countable plate (20-60 enterococci colonies) is obtained.*	11.5				
18) Are smaller sample sizes or sample dilutions used to minimize the interference of turbidity or for high bacterial densities? When analyzing smaller sample volumes (<20 mL),are 20-30 mL of PBS added to the funnel or is an aliquot of sample dispensed into a dilution blank prior to filtration?	11.6				
19) After the sample is filtered, are the sides of the funnel rinsed at least twice with 20-30mL portions of sterile PBS?	11.7				
20) Is the filter removed with sterile forceps and rolled onto the modified mEI agar to avoid the formation of bubbles?	11.8				

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21) Is the dish closed, inverted and incubated at 41 ± 0.5˚C for 24±2 hours? *Note: If the medium is plated in loose lid petri dishes, they should be incubated in a tight fitting container (e.g., plastic vegetable crisper) containing a moistened paper towel to prevent dehydration of the membrane filter and medium.*	11.8				
22) After incubation, are colonies that are ≥0.5 mm in diameter with a blue halo regardless of colony color counted and recorded as enterococci? Are filters containing, if practical, 20-60 enterococci colonies counted? *Note: When measuring colony size do not include the halo.*	11.9				
23) Is 2-5X magnification and a small cool white fluorescent lamp used for counting?	6.1, 6.2, 11.9				
24) Is enterococci count calculated as follows? Enterococci/100 mL = enterococci colonies counted X100 mL sample filtered	13.1				
25) Are multiple plates counted and calculated according to App. B?	13.2 App. B				
26) Are results reported as Enterococci per 100mL of sample?	13.3				
27) **Ongoing precision and recovery (OPR)** - Demonstration of ongoing control of the analytical system is achieved by routinely processing and analyzing spiked PBS samples. Does the laboratory analyze one OPR sample after every 20 field and matrix spike samples or one per week that samples are analyzed, whichever occurs more frequently? Are OPR samples accompanied by an acceptable method blank (§9.9) and appropriate media sterility checks (§9.11)? Is the OPR analysis performed as follows?	9.4				

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28) Is a 100-mL PBS sample spiked with *E. faecalis* ATCC #19433 according to the spiking procedure in §14? Spiking with laboratory-prepared suspensions is described in §14.2 and spiking with BioBalls is described in §14.3. Is each OPR sample filtered and processed according to the procedures in §11 and the number of enterococci calculated per 100 mL according to §13?	9.4.1				
29) Is the percent recovery (R) calculated for the OPR sample using the appropriate equation in §14.2.4.2 or 14.3.2 for samples spiked with laboratory-prepared spiking suspensions or BioBalls, respectively?	9.4.2				
30) Is the OPR result (percent recovery) compared with the corresponding OPR recovery criteria in Table 1? If the OPR result meets the acceptance criteria for recovery, method performance is acceptable and analysis of field samples may continue. If the OPR result falls outside of the acceptance criteria, system performance is unacceptable. In this event, is the problem identified by evaluating each step of the analytical process, media, reagents, and controls, correct the problem and repeat the OPR analysis?	9.4.3				
31) Ongoing Precision and Recovery (OPR) Acceptance Criteria Performance test / Lab-prepared spike acceptance criteria / BioBall™ acceptance criteria Ongoing precision and recovery (OPR) as percent recovery: 27% - 131% / 78% - 113%	Table 1				
32) As part of the laboratory QA program, are results for OPR samples charted and updated records maintained in order to monitor ongoing method performance? Does the laboratory develop a statement of accuracy for Method 1600 by calculating the average percent recovery (R) and the standard deviation of the percent recovery (s_r)? Is the accuracy expressed as a recovery interval from R - $2s_r$ to R + $2s_r$?	9.4.4				

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33) **Matrix spikes (MS) -** MS analysis are performed to determine the effect of a particular matrix on enterococci recoveries. Does the laboratory analyze one MS sample when disinfected wastewater samples are first received from a source from which the laboratory has not previously analyzed samples? Subsequently, do 5% of field samples (1 per 20) from a given disinfected wastewater source include a MS sample? Are MS samples accompanied by the analysis of an unspiked field sample sequentially collected from the same sampling site, an acceptable method blank (§9.9), and appropriate media sterility checks (§9.11)? When possible, are MS analyses accompanied by an OPR sample (§9.4), using the same spiking procedure (laboratory-prepared spiking suspension or BioBalls)? Is the MS analysis performed as follows?	9.5				
34) Are two, 100-mL field samples that were sequentially collected from the same site prepared. Does one sample remain unspiked and is analyzed to determine the background or ambient concentration of enterococci for calculating MS recoveries (§9.5.3)? Does the other sample serve as the MS sample and is spiked with *E. faecalis* ATCC #19433 according to the spiking procedure in §14?	9.5.1				
35) Are sample volumes Selected based on previous analytical results or anticipated levels of in the field sample in order to achieve the recommended target range of enterococci (20-60 CFU, including spike) per filter? If the laboratory is not familiar with the matrix being analyzed, are a minimum of three dilutions analyzed to ensure that a countable plate is obtained for the MS and associated unspiked sample? If possible, is 100-mL of sample analyzed?	9.5.2				

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36) Is the MS sample volume(s) spiked with a laboratory-prepared suspension as described in §14.2 or with BioBalls as described in §14.3? Are the unspiked and spiked field samples Immediately filtered and processed according to the procedures in §11? *Note*: When analyzing smaller sample volumes (e.g, <20 mL), 20-30 mL of PBS should be added to the funnel or an aliquot of sample should be dispensed into a 20-30 mL dilution blank prior to filtration. This will allow even distribution of the sample on the membrane.	9.5.3				
37) For the MS sample, is the number of enterococci (CFU / 100 mL) calculated according to §13 and the colony counts adjusted based on any background enterococci observed in the unspiked matrix sample?	9.5.4				
38) Is the percent recovery (R) calculated for the MS sample (adjusted based on ambient enterococci in the unspiked sample) using the appropriate equation in §14.2.4.2 or 14.3.2 for samples spiked with laboratory-prepared spiking suspensions or BioBalls, respectively?	9.5.5				
39) $R = 100 \times \frac{(N_s - N_u)}{T}$	14.2.4.2 14.3.2				
40) Is the MS result (percent recovery) compared with the appropriate method performance criteria in Table 2? If the MS recovery meets the acceptance criteria, system performance is acceptable and analysis of field samples from this disinfected wastewater source may continue. If the MS recovery is unacceptable and the OPR sample result associated with this batch of samples is acceptable, a matrix interference may be causing the poor results. If the MS recovery is unacceptable, are all associated field data flagged?	9.5.6				
41) Matrix Spike Precision and Recovery Acceptance Criteria based on data from spiked disinfected wastewater matrices Performance test — Lab-prepared Acceptance criteria — BioBall™ acceptance criteria Percent recovery for MS — 29% - 122% — 63% - 110%	9.5.7 Table 2.				

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42) Does the laboratory record and maintain a control chart comparing MS recoveries for all matrices to batch-specific and cumulative OPR sample results analyzed using Method 1600? These comparisons help laboratories recognize matrix effects on method recovery and help recognize inconsistent or sporadic matrix effects from a particular source.	9.5.8				

Notes/Comments